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Concentrating Ability of Lymphatic Vessels[1,2]

Aubrey Taylor, Harry Gibson

University of Mississippi Medical Center Department of Physiology and Biophysics
Jackson, Mississippi 39216

Summary

The fluid from implanted capsules was analyzed for plasma proteins and compared with fluid sampled from small lymphatics which drained the region of the implanted capsules. The plasma protein concentration of capsular and lymphatic fluids were not found to be statistically different, for capsules implanted for greater than one month. Capsules implanted for less than one month demonstrate a much higher total protein than lymphatic fluid. This difference is thought to be due to several factors: (1) length of time that capsule has been implanted, (2) inflammation surrounding capsule, (3) time lag between sampling of lymphatic and tissue fluids. If capsules were used that had been implanted for greater than 1-1/2 months, then capsular and lymphatic fluids are not statistically different. A model is presented which predicts that the small initial lymphatics could or could not concentrate plasma proteins depending on the fate of the leaked fluid. The concentrating ability of the initial lymphatics will depend on whether or not the fluid leaked from the initial lymphatic is large relative to the surrounding volume, the time course of protein diffusion to the vicinity of the lymphatic and the rate of fluid removal from the space immediately surrounding the lymphatic.

While there is a distinct possibility, since the forces exist, for a concentrating mechanism in larger lymphatic vessels, the data from implanted capsules indicate that we can assume that lymphatic vessels, especially in the subcutaneous region, contain plasma protein concentrations that are fairly representative of the tissue fluids of that region.

One of the most perplexing questions that one encounters when attempting to study the exchange of fluid at the capillary level is: What is the concentration of plasma proteins in tissue fluids? A classical investigation by *Drinker* and *Fields* suggested that lymphatic protein concentration was similar to that of tissue fluids sampled in the vicinity of the draining lymphatic (1). As a result, investigators since that time have simply sampled the fluid from a lymphatic which drains the region of interest and assumed that the concentration of protein in the lymphatic fluid represents that within the tissue fluid. However, the use of lymphatic protein concentration as a measure of the tissue protein concentration has been severely criticized by *Rusznyak, Foldi* and *Szabo*. These authors contend that a concentrating mechanism present in lymphatic vessels causes the lymphatic protein concentration to be higher than that in true tissue fluid (2).

It is a difficult problem to sample tissue fluid directly to measure protein concentration. The free fluid channels in the tissues are very, very small, and it is not yet possible to obtain fluid directly from normal tissue spaces. Needles and even capillary pipettes are much too large to sample tissue fluid except in the case of tissue edema. In 1961 we first implanted small perforated plastic capsules in subcutaneous tissues. Following a healing period of 4-6 weeks, a ml or so of fluid could easily be withdrawn from the interior of these capsules (3). Since the intracapsular fluid has been demonstrated by radioactive techniques to equilibrate with the surrounding tissue fluids, especially in regard to plasma proteins, the sampling of capsular fluid should be a very simple way to examine tissue fluid protein concentration (4).

The following paper will describe a series of experiments from our laboratory in which we have used the implanted capsule technique to obtain a tissue fluid sample. The plasma protein content of the capsular fluid was compared to the protein concentration of the fluid obtained from lymphatics draining the same region. Many physiologists feel that the lymphatic protein

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[2]This paper was presented at the International Society of Lymphology meeting in Tucson, Ariz. (1973).

is more concentrated than true tissue fluid protein because some concentrating mechanism is present either at the initial or larger lymphatic vessels. In order to gain more insight as to the possible site of the concentrating mechanism within the lymphatic system, we have developed two models placing the concentrating site at the initial lymphatic vessels and a third model placing the concentrating site at the larger lymphatic vessels.

Methods

Small perforated capsules were implanted in the hind paw region of 22 dogs. Following 4-6 weeks the animals were anesthetized with sodium pentobarbital (30 mg/kg), heparinized (10,000 units), and two small lymphatics well below the popliteal node were cannulated. The lymphatic fluid obtained from the small lymphatic vessels and plasma were analyzed for total protein and A/G ratios. Fluid was withdrawn through a 26 gauge needle from the interior of the capsular and analyzed for total protein and A/G ratios. Total proteins were measured by reading the refractive index using an American Optical refractometer and converting refractive index to plasma protein concentration (gm%) by a previously determined calibration curve: C/gm%) = $5 \cdot 10^2$ (refractive index - 1.3345). A milli-pore electrophoretic system was used to measure A/G ratios. Table I shows the results of these studies.

Table I Comparison of fluid withdrawn from implanted capsules and lymphatic vessels draining the region of the implanted capsules. Group I refers to all experiments and Group II refers to averages calculated by omitting data obtained in animals whose capsules were implanted for less than one month.

	Total Protein (gm%)	A/G Ratios	Differences (gm%) (C-L)
Group I (N = 22)			
Blood	6.5 ± 1.0 (35)*	1.22 ± .80 (24)	
Lymph	2.0 ± 0.7 (70)	1.59 ± 1.1 (38)	-0.46 + 1.00 (72)
Capsule	2.4 ± 0.9 (38)	1.68 ± 1.1 (21)	
Group II (N = 18)			
Blood	6.6 ± 0.9 (27)	1.13 ± 0.70 (21)	
Lymph	1.9 ± 0.6 (54)	1.41 ± 0.92 (33)	-0.14 + 0.85 (56)
Capsule	2.1 ± 0.8 (30)	1.56 ± 0.98 (19)	

*Mean ± SD, N refers to total number of animals, and numbers in parenthesis refer to number of measurements of each parameter. The last column (differences) refer to capsular total protein minus lymphatic total protein.

The average values shown in Table 1 are total protein and A/G ratios obtained from plasma, left hind paw lymphatic fluid, right hind paw lymphatic fluid and capsular fluid from either the right or left paw region. In each experiment two measuring periods of one hour each were used to obtain lymphatic fluid samples. This necessitated the use of two capsules, one for the first hour comparison of plasma proteins between capsular and lymphatic fluid and a second used for the second hour comparison. In some sample periods more than one capsule was used to sample capsular fluid.

The last column in Table 1 was calculated using the lymphatic and capsular total protein determinations for each hour period. A positive value indicates that lymphatic protein concentration is greater than capsular protein and a negative value indicates a lymphatic protein that is less than the protein concentration of capsular fluid.

Results

Group I in Table I shows the average results (± st. dev.) obtained from capsules and lymph in 22 different preparations. The total protein concentration of plasma proteins in the capsular

fluid is statistically different than that of lymphatic fluid (5 per cent level for an unpaired *t* test and .1 per cent level for a paired *t* test) when all the data is included in the analysis. In only 19 samples out of 72 was the lymphatic total protein higher than the capsular fluid protein, and in 10 of these instances the difference was equal to or less than .5 gm per cent. Also, the total protein in lymphatic and capsular fluids differed by only .55 gm% in 29 of the 72 pairings and of these determinations 9 showed less than .10 gm% difference. (3) The A/G ratios of lymph and capsular fluids are not statistically different (> 5 per cent level). If anything, the A/G ratios of capsular fluid appear to be slightly higher than that of lymphatic fluid. When experiments for which the capsules were implanted for less than 4 weeks are excluded from the statistical analysis (Group II), there is no statistical difference between total proteins or A/G ratios and the difference in total protein averaged -0.14 ± 0.85 gm%.

Discussion

The most probable cause of the larger protein value in capsular fluids from capsules implanted for less than one month is that there is always some inflammation surrounding the capsules. The appearance of the fluid within the high protein capsules is a reddish-yellow color. We collected several milliliters of this fluid and re-ran a calibration curve with known plasma proteins. The curve was identical with that determined with Tyrode's dilution of a standard protein solution. Therefore, there appears to be a real difference between capsular and lymphatic fluids with respect to plasma proteins in capsules implanted for less than one month. The capsular data in Table 1 had 10 values for which the total protein was greater than 3 gm%, whereas only 3 values of lymphatic fluids were in this range. In 8 of these high protein cases, the capsules had been implanted for less than one month. If these values were excluded from the statistical analysis (shown as Group II in table I) then the average capsular proteins and lymphatic proteins are not statistically different. We had hoped that the use of an implanted capsule for obtaining a tissue fluid sample would prove to be experimentally useful; but, the data in Table 1 indicates that capsules implanted for less than one month can have plasma proteins that are much higher than lymphatic proteins. However, for capsules implanted for greater than one month the technique appears to measure a good cross-section of the tissue that is drained by the lymphatic vessel.

Another possible source of the difference observed in the total protein data is lag-time between lymphatic and capsular fluid determinations. Lymph flow in these studies was extremely low, averaging only .0015 ml/min. We did not propel the lymph by limb movement during any experimental measuring period; however, we did stroke the lymphatic proximal to the cannula to insure that the lymphatic cannula was patent prior to the beginning of our experimental measurements. Perhaps even this slight propulsion of lymphatic fluid disturbed the resting steady-state forces of the tissues and resulted in a more diluted lymphatic fluid relative to capsular fluid.

Since the capsular protein is slightly (although not statistically different for capsules implanted for greater than one month) higher or equal to lymphatic protein, then any concentrating mechanism of the lymphatics would be masked, and any concentrating mechanism, if it exists, would be difficult to describe using protein data from implanted capsules.

Concentrating Ability of the Initial Lymphatic. One possible concentrating site in the lymphatic system is the small initial lymphatics. These small terminal lymphatic vessels initially fill with tissue fluid which enters their lumen through large endothelial gaps. The fluid is then propelled from the initial lymphatic vessels into collecting and larger lymphatics by either tissue compression on the lymphatic vessels or some downstream pressure gradient caused by an intrinsic lymphatic pump.

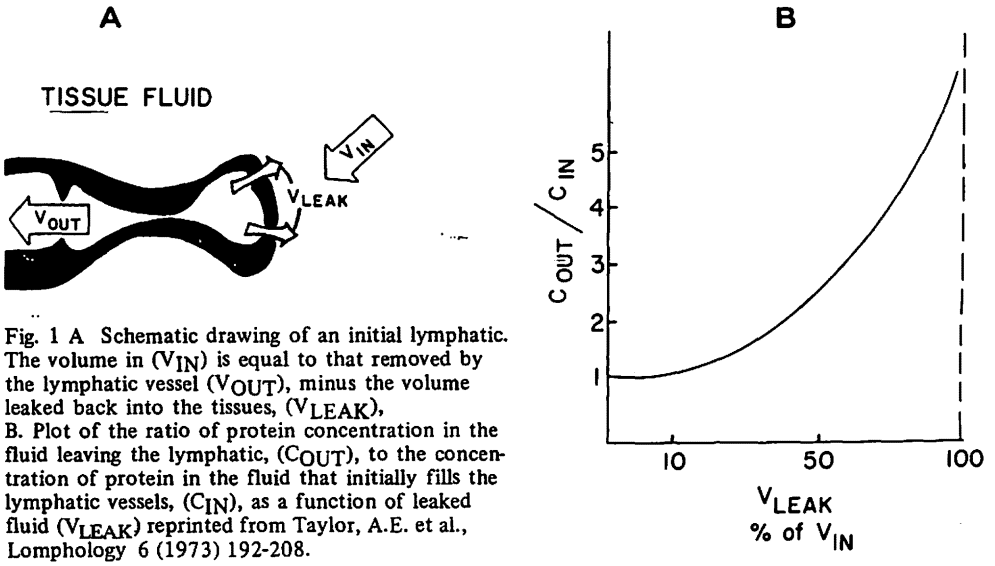

Fig. 1 A Schematic drawing of an initial lymphatic. The volume in (V_{IN}) is equal to that removed by the lymphatic vessel (V_{OUT}), minus the volume leaked back into the tissues, (V_{LEAK}),
B. Plot of the ratio of protein concentration in the fluid leaving the lymphatic, (C_{OUT}), to the concentration of protein in the fluid that initially fills the lymphatic vessels, (C_{IN}), as a function of leaked fluid (V_{LEAK}) reprinted from Taylor, A.E. et al., Lomphology 6 (1973) 192-208.

Concentrating Mechanism Located at Initial Lymphatic Vessel. Casley-Smith has proposed a model in which the initial lymphatic concentrates the plasma protein within its lumen and then refills by an osmotic force across its wall. The osmotic pull is through large endothelial junctions and the net result is a fluid leaving the initial lymphatic that is concentrated above that in the tissues. As the lymph fluid courses through the larger lymphatics, then fluid is withdrawn from the surrounding tissues such that the concentrated fluid in the large lymphatics is diluted to equal that which is in the tissue spaces (5).

Fig. 1 is a schematic drawing of an initial lymphatic that is used here to devlop a simple mathematical model of lymphatic and tissue protein concentration. Let us assume that during the dilatation cycle a volume V_{IN} enters the initial lymphatic from the tissue spaces. Then during the contraction cycle, a volume is lost back into the tissue through the leaky pores equal to a volume V_{LEAK}. The amount of volume moved forward by the lymphatic is V_{OUT}. The relationships between the volumes, quantity of protein (Q_i), and concentration (C_i) in this model are:

$$V_{OUT} = V_{IN} - V_{LEAK}$$

$$Q_{OUT} = Q_{IN} \text{ (if we assume no protein in leak as postulated by Casley-Smith)}$$

or

$$V_{OUT}\, C_{OUT} = C_{IN}\, V_{IN}$$

$$V_{OUT}\, C_{OUT} = C_{IN}\, (V_{OUT} + V_{LEAK})$$

$$C_{OUT} = \frac{(V_{OUT} + V_{LEAK})\, C_{IN}}{V_{OUT}}$$

or

$$\frac{C_{OUT}}{C_{IN}} = 1 + \frac{V_{LEAK}}{V_{OUT}} \qquad \ldots (1)$$

Fig. 2 is a plot of C_{OUT}/C_{IN} as a function of V_{LEAK}. As the leak increases, the ability of the lymphatic to concentrate increases. Note that for a 20 per cent leak, the lymphatic fluid is

concentrated to 25 per cent above the concentration of the fluid which has entered the lymphatic. If the fluid that leaks from the lymphatic is rapidly absorbed by capillaries adjacent to the initial lymphatic, then the next filling cycle presents a fluid to the lymphatic that is identical to that which first filled the lymphatic. However, the volume pushed out of the initial lymphatic and returning to the tissues must move through the interstitium. If the tissue is not edematous, then the conduction through the tissues is low and unless the capillary is close to the lymphatic it is doubtful that the lost fluid will be removed. A concentrating mechanism would then be present at the initial lymphatic as proposed by *Casley-Smith* (5) if the volume is rapidly removed.

No Concentrating Mechanism Located at Initial Lymphatic Vessel. If the volume moved away from the tissue by lymphatic vessel is replaced by some tissue fluid with a protein concentration C_{TISS} and the leaked volume is not rapidly removed by the capillary system surrounding the initial lymphatic, the fluid within the initial lymphatic can only be concentrated to a value equal to C_{TISS}, regardless of the leakage volume.

The volume of the leak that enters the tissue immediately surrounding the initial lymphatic will finally be mixed with a volume that is equal to V_{OUT} with a protein concentration of C_{TISS}. The protein concentration in the immediate vicinity of the initial lymphatic equals

$$C_{IN} = \frac{V_{OUT} \cdot C_{TISS}}{V_{OUT} + V_{LEAK}}$$

Substituting this into Equation 1 yields

$$C_{OUT} = \frac{(V_{OUT} + V_{LEAK})}{V_{OUT}} \cdot \frac{(V_{OUT}\ C_{TISS})}{V_{IN}}$$

Now $V_{OUT} + V_{LEAK} = V_{IN}$

$$C_{OUT} = C_{TISS}$$

Therefore, the concentration in the lymphatic could easily represent some average protein concentration in the tissue that is responsible for movement into and out of the capillary. The concentration of protein in the immediate vicinity of the lymphatic would be less than that of the lymphatic or the tissue protein concentration active at the capillary wall. For this particular model to be operative in tissues the volume leaked from the initial lymphatic must stay in the vicinity of the initial lymphatic and mix with some tissue protein. If capillaries in the immediate vicinity of the initial lymphatic carry away the leaked fluid, then the lymphatic vessel can concentrate plasma proteins above tissue proteins.

The above analyses do not consider the diffusion of protein from the surrounding tissue into the volume ejected by the initial lymphatic. The final concentration of protein in the lymphatic is a very complex function of diffusion distances to lymphatics as well as lymphatic leakage. It is our opinion that lymphatics in subcutaneous tissue do not concentrate to any great extent, but a more extensive model is now in progress to further examine the regulation of this important tissue force.

Concentrating Ability of the Larger Lymphatics. What about the possibility of a concentrating mechanism for plasma proteins in the larger lymphatic vessels? The lymph flow in subcutaneous tissue is normally very, very low, and yet, there is an average intra-lymphatic pressure which is above tissue pressure (6). This pressure difference would provide a driving force to push fluid outward through the lymphatic pores into the tissues. If the proteins do not leak, this obviously would result in a more concentrated lymphatic fluid within the more distal lymphatics. However, there are many physiological properties of large lymphatics which we do not

presently understand. In order to formulate a working model it is necessary that we obtain new experimental information for the following problems: (1) What are the filtration properties of the larger lymphatics – that is, how much fluid moves across the lymphatic wall for a given pressure head? (2) What type of capillaries and/or small lymphatic system surrounds the larger lymphatics? The capillaries and/or small lymphatics in the vicinity of the larger lymphatics must carry away the volume that is moved back into the tissue surrounding the large lymphatics or there can be no concentrating mechanism in the large lymphatic vessels. When fluid leaves the larger lymphatics, the tissue pressure in the immediate vicinity of the lymphatic will rise and this force will then oppose the filtration forces present in the capillaries adjacent to the large lymphatics. The net result will be: (a) Either direct absorption into the capillaries surrounding the large lymphatics, or (b) drainage of the leaked fluid by the small lymphatic system of the tissue immediately surrounding the large lymphatics. (3) What would be the maximum over-all effect one could expect from a concentrating mechanism present in the large vessels? The lymphatic pressure minus tissue pressure certainly provides a net driving force to push fluid out of the large lymphatics. Theoretically, fluid would leave the lymphatic vessel until the intra-lymphatic and tissue forces are at an equilibrium state. We can calculate this equilibrium state by assuming several parameters: (1) Average intra-lymphatic pressure equals to 5 mmHg. (2) Interstitial fluid pressure equals to -6 mmHg. The net driving force is equal to 11 mHg; therefore, the intra-lymphatic protein osmotic pressure could theoretically rise to 11 mgHg above tissue osmotic pressure. This is certainly impossible in subcutaneous tissue (see Table 1). Another approach to the problem is to assume tissue protein is equal to intra-lymphatic proteins and that the filtration coefficient of the large lymphatics is equal to that of skeletal muscle (7). Thus initially,

Leaked volume = .02 (Lymphatic pressure – tissue pressure)
= .22 ml/min/100 gm.

Now in subcutaneous tissue, lymph flow is equal to .006 ml/min/100 gm (4); therefore, the possible volume movement across the lymphatic wall could be 36 times that of lymph flow. It is possible that the capillary net filtrate could be several times that which appears in the final lymphatic. Even if the filtration coefficient of the lymphatic walls was say only 2/36 of the skeletal muscle filtration coefficient, twice as much fluid could leave the capillary as compared to that which finally drains the lymphatic system and the lymphatic vessels could concentrate the protein within their lumen considerably above that in the tissues. As intra-lymphatic protein concentration increases, the volume flow crossing the lymphatic per unit time will decrease as the fluid is propelled forward in the large lymphatic system.

The difference in capillary filtrate and lymph flow could certainly be possible since Intaglietta has shown that in certain tissues there is always a large driving force out of small capillaries, i.e., capillary pressure is always larger than plasma colloid osmotic pressure (8). Also, *Johnson* (personal communication) has some convincing data concerning this problem that also suggests a higher net capillary filtration than that accounted for by lymph flow.

Casley-Smith's model predicts that fluid should be pulled into the large lymphatics due to the increased plasma protein content of the fluid. The intra-lymphatic pressure minus tissue pressure would most likely vary between 7-11 mmHg. For filtration to occur into the large lymphatics, then the colloidal osmotic pressure of the plasma proteins within the lymphatics must be greater than 9 mmHg + π_{TISS}, which would require that the osmotic pressure in the initial lymphatic be several times that of the tissue. Zweifach has recently demonstrated that the initial lymphatic pressure is equal to 0 mmHg and is not oscillatory (9). It is therefore highly unlikely that the initial lymphatic concentrates plasma proteins to a level sufficient to cause absorption into large lymphatics since any calculated filtration force is relatively large and favoring movement out of the larger lymphatics.

It is quite difficult to see how any significant lymphatic concentrating mechanism could be possible in subcutaneous tissue. However, other tissues particularly those with high lymphatic protein could possibly have significant protein concentrating mechanisms.

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Aubrey E. Taylor, University of Mississippi Medical Center, Department of Physiology and Biophysics Jackson, Mississippi 39216

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Functional Anatomy of the Lymphatic Fluids and Pathways

Simon Rodbard, Paula Feldman

City of Hope Medical Center, Department of Cardiology, 1500 East Duarte Road, Duarte, California 91010

Summary

Summary

The present sets of studies indicate that the fibrous capsule which encloses each tissue module divides the interstitial fluids into an intracapsular pool, and an extracapsular pool. Fluid that filters out of the capsules into the extracapsular clefts is the source of the lymph. Because of the limited permeability of the capsular barrier the composition of lymph differs from that of the capillary ultrafiltrate. Lymphatic vessels are means for the drainage of the extracapsular fluids and other materials. This approach differentiates two entirely separate types of edema: an intracapsular dependent pitting edema and an extracapsular generalized non-pitting edema. Three sets of experiments that support the foregoing hypothesis are briefly presented.

Our studies have led to some new perspectives related to the anatomy, physiology and pathology of the lymph and the lymphatic vascular system (1). The implications inherent in our approach provide for re-assessment of the interstitial fluid pool, the anatomical capsules which separate capillary ultrafiltrates from prelymphatic fluids, and related problems.

Current concepts hold that there is only a single interstitial fluid, from which the lymphatic system extracts a minuscule, nearly vanishing volume for return to the blood stream. Thus, osmotic and hydrostatic forces return approximately 99.9 percent of the capillary ultrafiltrate at the downstream end of the blood capillaries. The remaining one part in a thousand of the